THE COEXISTENCE OF ANTIBODY AND ANTIGEN IN THE BODY *

PLATES 24 AND 25

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INTRODUCTION

The most striking feature of the immune reaction consists in the production of specific protective substances, known as antibodies, in response to the presence within the body of a foreign protein, the so-called antigen. These factors, indeed, have been identified, but the finer details of the mechanism of the defensive process are almost entirely obscure. The theory originally propounded, and even now generally entertained, was based on the high degree of affinity which antibody has for antigen. As rapidly as the organism could produce these protective substances, they were supposed to attack the antigen, and to neutralize or destroy it. After this process had been brought to completion, so that no free antigen remained in the body, free antibody was supposed to accumulate, and was then demonstrable as such in the blood. According to this view, the latent period which followed the introduction of antigen into an organism, and which preceded the demonstrable presence of free antibody in its blood, was occupied by the neutralization of antigen by antibody. The succeeding period, the so-called positive phase, characterized by the presence of free antibody, was taken to denote the complete neutralization of the antigen.

The validity of this theory was seriously threatened by a series of observations which seemed to indicate the coexistence of antigen and antibody within the blood over considerable periods of time.

Uhlenhuth and Weidanz,[1] and others, have noted the fact that the sera from two different rabbits, immunized against the same protein, when mixed produce a precipitate. Eisenberg[2] showed that when antigen and antibody are mixed in the test tube, the resulting precipitate does not carry down the total amount of either factor, but that both may be demonstrated in the

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[1] Technik u. Methodik des biologischen Eiweissdifferenzierungsverfahren, 1909.

[2] Centralbl. f. Bakteriol., I, O., 1903, 34, p. 259.

supernatant fluid. These two striking phenomena were interpreted as analogous; the mutual precipitation of two immune sera was regarded as evidence of the presence in both, or in one of them, of antigen in addition to antibody. Those who have believed that immunologic reactions are simply exemplifications in another field of the laws of mass action in chemistry, welcomed these facts as evidence of the correctness of their views. To Ehrlich and his followers, however, the physicochemical interpretation of the phenomena of immunization has never appeared satisfactory. In consonance with this standpoint, v. Dungern[3] has explained the observations of Linossier and Lemoine,[4] and of Eisenberg, on the basis of a fresh hypothesis. He maintains that an animal serum is not a single homogeneous antigen or protein, but a complex, or mixture, of a large number of such antigens, many of which are quite dissimilar in character. To some of these antigens it is conceivable that the antibody reaction is prompt and marked, while to others it is relatively deficient. For instance, an animal injected with a foreign serum may contain at one and the same time antigens of the latter group and antibodies to the former group. Such a situation would permit of the reactions described by these authors, whereas it by no means corresponds to their interpretation; namely, that antigen and its specific antibody may coexist in the blood. Von Dungern's theory has not been demonstrated to be true, nor has it, on the other hand, been disproved; so that the facts must still be accepted, with the reservation that their interpretation is not absolutely beyond question.

Recently this problem in the mechanism of immunity has been approached with the help of new methods by Weil.[5] In the first place, he has shown that antigen and antibody may be demonstrated at the same time in the sera of persons who have received large doses of therapeutic horse serum. In this respect, therefore, the reaction of human sera is analogous to that of the sera of the laboratory animals.

In other papers Weil[6] has studied the problem of the coexistence of antigen and antibody, not only in the blood, but also in the cells of the animal. His studies have been based entirely on the use of the methods of anaphylaxis. An interesting feature of this method consists in the fact that a positive result demonstrates the presence of antibody in a form and in amounts which are physiologically effective. The technic employed by Weil, including the use of an immune body as antigen, has been adapted to the present study, and will subsequently be described in detail. The conclusions reached by him are as follows:

After the subcutaneous administration of a large dose of a foreign serum to a guinea-pig, this serum persists demonstrably for a period of about 2 weeks, not only in the blood, but in the cells of the guinea-pig. During the latter part of this period the guinea-pig develops specific antibodies to the foreign serum, the presence of which can be demonstrated in the cells of the animal. Later on, these antibodies are demonstrable in the blood. Thus, the reaction of an organism to a foreign protein is shown to be characterized by 4 factors, all of which, except the last named, are known to coexist for a certain period of time in the body; namely, antigen in the cells, antigen in the blood, antibody in the cells, and antibody in the blood.

[3] Centralbl. f. Bakteriol., I, O., 1903, 34, p. 355.
[4] Compt. rend. Soc. de biol., 1902, 54, p. 85.
[5] Proc. Soc. Exper. Biol. and Med., 1914, 12, p. 37.
[6] Jour. Med. Research, 1914, 30, p. 299.

These factors, moreover, exert a marked influence on one another, inasmuch as they materially modify the vital reactions of the animal. A guinea-pig, the cells of which contain both antibody and antigen, responds to the addition of fresh antigen in much milder fashion than does an animal in which antibody alone is present. In the course of time, antigen disappears from the cells and from the blood, leaving only antibody, which eventually tends to disappear from the blood, but is demonstrable for an almost indefinite period in the cells. In a subsequent publication Weil[7] made use of the methods of passive sensitization to demonstrate the coexistence of antigen and antibody in the organism. As these particular studies do not touch the special problem considered in this paper, they require no further description.

Previous to the work analyzed, the study of the mechanism of immunity involved the consideration of only one factor; namely, the presence of antibodies in the serum. It seemed of importance to make a further study of the 4 factors described, and to determine, if possible, additional data as to their mode of interaction.

The problem undertaken in the present study to a certain extent involved a repetition of the work reported by Weil, with certain differences. In the first place, a different antigen was selected. Weil had made use of the serum of rabbits immunized against horse serum. This antigen is open to the objection that the uterus of a guinea-pig highly sensitized to rabbit serum may react to horse serum. This condition was pointed out by Weil, entailing as it did certain special precautions. Nevertheless, in spite of the fact that disturbance from this factor was excluded by the experimental conditions, it seemed advisable in the present study, to select as antigen a serum containing antibodies not open to this objection. For this purpose, use was made of the serum of a rabbit highly immunized against egg albumin. There is never any question of cross sensitization as between egg albumin and rabbit serum.

Altho Weil had succeeded in demonstrating the coexistence of 3 factors in the mechanism of immunity, and had assumed the presence of the 4th factor (antibody in the serum) as already fully demonstrated by previous investigations, he did not attempt a careful analysis of the time-relationships of these factors. It is of interest to know whether the appearance of antibody in the cells antedates the appearance of the same substance in the blood. It is of considerable importance to know whether the presence of antibody in the serum does, or does not, indicate the complete disappearance of antigen from the cells. In the present study an attempt has been made to approach the solution of these questions, with, however, the distinct realization that

[7] Ztschr. f. Immunitätsf., 1914, 23, p. 1.

the methods at our disposal permit of the detection of only fairly large amounts either of antigen or of antibody.

TECHNIC AND METHODS

The technic has followed, in most respects, the methods described by Weil. In one detail of Dale's method, however, a modification has been adopted. In case of a low degree of sensitization, the uterus responds to the application of large amounts of antigen, while smaller amounts are ineffective. In testing for the presence of antibody, therefore, it is advisable to use the largest amount of antigen that will not cause a muscular contraction of the normal control uterus. In order to follow this technic, preliminary titrations were made with egg albumin and with rabbit serum, the results of which are included in this paper. It was found possible to employ in the tests amounts considerably larger than those used by Weil. As a result, passive antibody (antigen) has been shown to persist longer and active antibody to occur earlier, than was possible in previous experiments. At the same time, the period of overlapping of antigen and antibody has been extended.

Another variation of technic was attempted, with the object of demonstrating the persistence of minute traces of antigen (passive antibody) in the blood of the guinea-pig. For this purpose, Weil exsanguinated the animal and used the entire blood to sensitize a 2nd guinea-pig passively. Obviously, the success of this experiment requires the persistence of considerable amounts of antigen. The attempt was therefore made to demonstrate the antigen by testing the blood of the guinea-pig against the uterus of a guinea-pig sensitized to the antigen in question. Thus, if it is desirable to test for the persistence of antigen in a guinea-pig which has received an injection of the serum of a rabbit immunized against egg albumin, the blood of that guinea-pig is added, in proper amount, to the uterine preparation of a guinea-pig sensitized towards rabbit serum (Figs. 9 and 10). A contractile response reveals the persistence of the rabbit component in the blood of the 1st guinea-pig. The method, which had been previously used, is sensitive and accurate.

Preparation of Immune Serum.—Rabbits were immunized against egg albumin by daily, or almost daily, intravenous inoculation of gradually increasing doses of the antigen. A typical report is given in Table 1. On January 25, a small quantity of blood was taken from the ear vein, and the serum injected as shown in Table 2.

TABLE 1

IMMUNIZATION OF RABBIT 564

Date of Inoculation	Dose in c.c. of Egg Albumin
Jan. 9	0.2
Jan. 11	0.4
Jan. 12	0.4 and again after ½ hour 0.4
Jan. 13	0.4 and again after ½ hour 0.6
Jan 15	0.6
Jan. 18	0.6 and again after ½ hour 0.4

TABLE 2

RESULTS OF SENSITIZATION OF GUINEA-PIGS TO EGG ALBUMIN

Guinea-pig	Jan. 25 (Rabbit serum 564 injected intraperitoneally)	Jan. 27 (Egg albumin injected intravenously)	Result
518	0.25 c.c.	0.5 c.c., 50% egg albumin (diluted with salt solution)	Died immediately
519	0.1 c.c.	0.5 c.c., 50% egg albumin (diluted with salt solution)	Died immediately

Table 2 shows that the sensitizing dose (or titer) of this serum is 0.1 c.c. or less. A serum of lower titer was never used. The guinea-pigs used in the experiments to be outlined, usually received 3 c.c. of the immune serum intraperitoneally, in other words, 30 sensitizing doses, or more.

Methods of Determining the Presence of Antigen and Antibody.—The essential feature in the experiments is the use of an immune serum as antigen. Thus, guinea-pigs were prepared by the injection of 3 c.c. of rabbit serum immunized against egg albumin. The animals were killed by exsanguination at intervals varying between 9 and 24 days after injection, and tests were made at once for intracellular antigen and intracellular antibody. The uteri were suspended in a Dale apparatus. Antigen was demonstrated by uterine contraction following the addition of egg albumin to the Locke's fluid bathing the uterus. Antibody was demonstrated by uterine contraction following the addition of rabbit serum. The reaction of the uterus to egg albumin indicates the presence of rabbit serum in the uterine cells, for the guinea-pig cells contain antibodies for egg albumin only by virtue of the absorption of serum immunized against egg albumin. The reaction of the uterus to rabbit serum reveals the production by the guinea-pig of antibodies to the injected rabbit serum. Briefly, then, the response to egg albumin indicates the presence of antigen, and the response to rabbit serum, of antibody.

The demonstration of circulating antigen and circulating antibody involves the use of similar methods. The serum obtained by exsanguination of the guinea-pigs inoculated with rabbit serum versus egg albumin, is injected into normal virgin pigs intraperitoneally. After 3 or 4 days these pigs are killed and the uteri are tested for antigen and antibody, as outlined. Certain differences involved in the demonstration of circulating antigen and antibody as compared with the demonstration of the cellular elements, will be considered more fully with the description of the individual experiments.

Before these experiments could be begun, certain preliminary tests were necessary. The uterus of a normal guinea-pig will contract on the addition of any serum, provided that a sufficient amount be employed. It was therefore necessary to determine the largest amounts of egg albumin and rabbit serum that could be used without causing a contraction in a normal uterus. It was found that as much as 3 c.c. (Fig. 1) and 5 c.c. (Fig. 2) of egg albumin could be added without causing contraction in a normal uterus. Amounts of rabbit serum up to 0.5 c.c. (Fig. 1) consistently failed to cause contraction. As a rule, 0.7 c.c. and 1 c.c. also failed to elicit a response, but because of an occasional contraction when the latter quantity was used, 0.5 c.c. of rabbit serum was decided upon as the largest quantity to be employed in testing for the presence of antibody.

THE PRESENCE AND PERSISTENCE OF ANTIGEN IN THE CELL

The following is the method by which cellular antigen is demonstrated. Guinea-pigs are injected, intraperitoneally, with 3 c.c. of rabbit serum immunized against egg albumin. At varying intervals of time after the injection, the animals are exsanguinated and the uteri suspended in a Dale apparatus. Contraction following the addition of egg albumin to the fluid surrounding the uterus, indicates the presence of antibodies for egg albumin in the uterine cells; the presence, in other words, of the rabbit serum component—antigen (Fig 3).

TABLE 3
CELLULAR ANTIGEN

Guinea-pig	Days After Injection	Reaction Present + or Absent 0	Amount in c.c. of Egg Albumin Used to Elicit Reaction
104	9	+	0.1
120	9	+	0.3
121	11	+	0.4
123	11	+	1.8
714	11	+	0.8
685	12	+	0.75
127	14	+	1.5
525	17	+	1.2
719	18	0	2.0
529	21	0	1.8
526	24	0	1.5
...	..	.	...
103	12	0	0.2
122	13	0	0.3

These experiments cover a period from the 9th to the 24th day after the injection of rabbit immune serum. Antigen has been demonstrated from the 9th (Figs. 3 and 4) to the 17th day (Fig. 5); after the 17th day the antigen disappears from the cell, as indicated by the fact that reactions with large doses of antigen could not be obtained on the 18th, 21st, and 24th days. Altho not tested previous to the 9th day, it is evident that antigen must be present from the 2nd day after injection, as passive sensitization occurs within 24 hours. It is important to note that the demonstration of antigen requires increasingly large amounts of egg albumin the longer the interval after the sensitizing injection. For example Table 3 (see also Figs. 3, 4, and 5) shows the following: Uterus of Guinea-pig 104, 9 days after injection, reacted to 0.1 c.c. egg albumin. Uterus of Guinea-pig 103, 12 days after injection, failed to react to 0.2 c.c. egg albumin. But uterus of Guinea-pig 685, also 12 days after injection, responded to 0.75 c.c. egg

albumin. Further, uterus of Guinea-pig 525, 17 days after injection, responded to 1.2 c.c. egg albumin, whereas uterus of Guinea-pig 719, 18 days after injection, failed to respond to 2 c.c. egg albumin. After the 17th day, then, antigen apparently disappears from the cell, or, at any rate, can no longer be demonstrated in the cell by methods which were effectual before that time.

Table 3 presents the results of a series of tests in detailed form.

THE OCCURRENCE AND PERSISTENCE OF ANTIBODY IN THE CELL

The preparation of the guinea-pigs for the demonstration of cellular antibody is identical with that described in the previous section, as the same uteri which were tested for cellular antigen were used to demonstrate the presence of antibody. Rabbit serum was added to the Locke's fluid, and a uterine contraction indicated the presence in the cell of antibodies to rabbit serum.

The experiments cover a period from the 9th to the 24th day after injection, and antibody has been demonstrated during this entire time (Table 4 and Figs. 3 and 4). The antibody was presumably present even before the 9th day, but as no experiments were performed before that time, the earliest demonstrable appearance of cellular antibody is not definitely known. Cellular antibody persists after the 24th day, probably indefinitely. The experiments of Anderson, referred to in the first part of this paper, indicate the persistence of antibodies during the entire life of the guinea-pig. Table 4 presents in detail the results of these tests.

TABLE 4

CELLULAR ANTIBODY

Guinea-pig	Days After Injection	Reaction Present +	Amount in c.c. of Rabbit Serum Used to Elicit Reaction
104	9	+	0.1
120	9	+	0.5
121	11	+	0.3
123	11	+	0.3
685	12	+	0.3
122	13	+	0.3
127	14	+	0.5
525	17	+	0.4
719	18	+	0.3
529	21	+	0.5
526	24	+	0.3

THE PERSISTENCE OF CIRCULATING OR FREE ANTIGEN

The method (another method will be described in the latter part of this paper) of demonstrating circulating antigen is as follows: Guinea-pigs are prepared as described; that is, 3 c.c. of rabbit serum immunized against egg albumin are injected intraperitoneally. After varying intervals the pigs are exsanguinated, and the serum obtained is injected intraperitoneally into another guinea-pig. The latter pig is killed after 3 or 4 days, and the uteri are tested for the presence of antigen (by the reaction to egg albumin) and for the presence of antibody (by the reaction to rabbit serum). As an example of the method, one such experiment will be described in detail.

Guinea-pig 104 received 3 c.c. of rabbit serum (Rabbit 291, highly immunized against egg albumin). After an interval of 9 days, this guinea-pig was killed by exsanguination, and the uteri were tested for cellular antigen and antibody (Fig. 3), both of which were demonstrated. The 2 c.c. of serum, obtained by exsanguination, were injected intraperitoneally into Guinea-pig 65. Three days later Guinea-pig 65 was killed and the uteri were tested for antigen and antibody. Fig. 6 shows a marked reaction to egg albumin, indicating the presence of a rabbit serum component (antigen) in the blood of Guinea-pig 104. No reaction was obtained on the addition of rabbit serum, a result indicating that on the 9th day antibody had not appeared in the circulating blood in an amount sufficient to confer passive sensitization.

Briefly, then, Guinea-pig 104 demonstrates that on the 9th day antigen persisted in both cells and circulation, and that antibody had been produced and was demonstrable in the cells, but had not as yet appeared in the blood.

TABLE 5

Free or Circulating Antigen

Guinea-pig	Days After Injection	Reaction Present + or Absent 0	Amount in c.c. of Egg Albumin Used to Elicit Reaction
104	9	+	0.8
120	9	+	0.4
121*	11	+	2.0
685	12	+	3.0
525	17	+	1.8
719	18	0	2.5
529	21	0	1.5

* Presence of free antigen indicated also by the reaction of 0.5 c.c. of the serum of Guinea-pig 121 on uterine preparation of Guinea-pig 1260, which was actively sensitized against rabbit serum.

This method of demonstrating circulating antigen has very definite limitations. It is not satisfactory for showing small amounts, as enough must be present to sensitize a guinea-pig passively. The results depend to a considerable degree on the quantity of blood recov-

ered on exsanguination; if the yield is small, the demonstration of the immune body may fail. These factors explain the irregularity of the results.

The antigen is of course present in relatively large amounts in the blood during the 1st week after sensitization. Its persistence can be demonstrated for 17 days (see Figs. 7 and 8). Tests made on the 18th and 21st days after injection fail, however, to show the presence of circulating antigen. Table 5 presents details of these experiments.

THE OCCURRENCE AND PERSISTENCE OF CIRCULATING ANTIBODY

The method is identical with that just described for the demonstration of circulating antigen; rabbit serum instead of egg albumin, is used to elicit the reaction. The same limitations obtain in the demonstration of circulating antibody as were just mentioned with reference to free antigen, since the results depend in considerable degree on the amount of blood recovered by exsanguination.

Antibody does not appear in the circulation until the 14th day (Table 6), but is constantly present after that time (Fig. 8). The experiments cover a period of only 21 days; antibody is known to persist in the blood for a longer period, but the extreme limit of its occurrence has not been tested by this method.

TABLE 6

FREE OR CIRCULATING ANTIBODY

Guinea-pig	Days After Injection	Reaction Present + or Absent 0	Amount in c.c. of Rabbit Serum Used to Elicit Reaction
104	9	0	0.5
120	9	0	0.4
121	11	0	0.5
685	12	0	0.5
127	14	+	0.5
525	17	+	0.5
719	18	+	0.55
529	21	+	0.5

INTERRELATIONSHIP OF FOUR FACTORS

The time relationship of the 4 factors may be best appreciated by roughly grouping the appearance and disappearance of each of them into 4 periods of 1 week each (Table 7 and Chart 1). In the 1st week antigen is present, both in the blood and in the cells; in the 2nd week antibody appears in the cells, and the cellular and circulating antigen persist; in the 3rd week, antibody appears in the blood and antigen disappears from both cells and blood; antibody persists in the cell; in the 4th week, antibody is present both in the cells and in the blood, while antigen is no longer demonstrable in either.

For a period of more than a week antigen and antibody coexist in the cells. For several days antigen and antibody coexist in the circulation. During a short period of a few days all 4 factors are demonstrable together. These relations are more convincingly demonstrated by data illustrating the occurrence of all 4 factors in individual animals. Thus, Guinea-pig 525 received 3 c.c. of rabbit serum versus egg albumin. Seventeen days later the uteri of this animal showed the presence of both antigen and antibody (Fig. 5). The serum recovered by exsanguination of Guinea-pig 525 was injected into Guinea-pig 590. Four days later this animal was killed; Figure 8 shows the presence of antigen and antibody in its uterine cells. This indicates that antigen and antibody were present in the serum of Guinea-pig 525, which was used to sensitize Guinea-pig 590. Thus, the coexistence of all 4 factors was demonstrated in one animal.

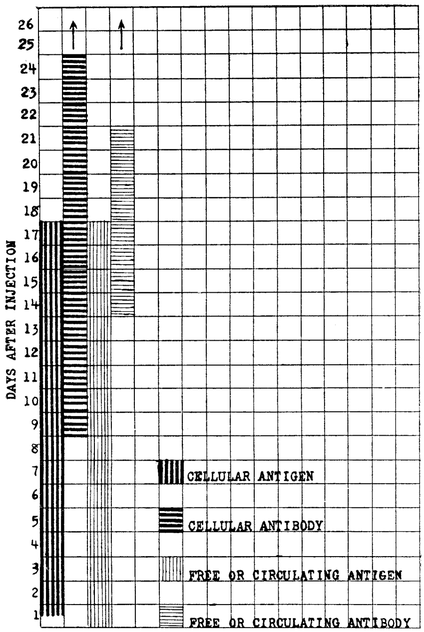

Chart 1. The persistence of cellular and circulating antigen and the occurrence of cellular and circulating antibody. Cellular antigen may be assumed to be present before the 9th day, tho no test was made for it by this method. No test was made for cellular antibody before the 9th or after the 24th day, and none for free or circulating antibody after the 21st day. Between the 14th and 17th days, the lines indicating the presence of cellular antigen and antibody and circulating antigen and antibody overlap; in other words, for a short period of time (14 to 18 days), these 4 factors in immunity coexist.

Table 7 presents these data in detailed form. Chart 1 gives a graphic illustration of the facts.

TABLE 7

APPEARANCE, PERSISTENCE, AND DISAPPEARANCE OF ANTIGEN AND ANTIBODY

Guinea-pig	Days After Injection	Cellular Antigen	Cellular Antibody	Circulating Antigen	Circulating Antibody
104	9	+	+	+	0
120	9	+	+	+	0
121	11	+	+	+	0
685	12	+	+	+	0
127	14	+	+	?	+
525	17	+	+	+	+
719	18	0	+	0	+
529	21	0	+	0	+
526	24	0	+		

DEMONSTRATION OF FOREIGN ANTIGEN IN THE BLOOD

Altho not absolutely essential to the present study, two other types of experiments will be outlined.

The first is a method for demonstrating circulating antigen in small amounts of serum. It will be remembered that the demonstration of circulating antigen was rendered difficult and uncertain because a large amount of serum was required whereas the amounts that actually were recovered on exsanguination of the sensitized guinea-pig were variable. The method to be described demands only 0.5 c.c. serum. The rationale of the procedure is as follows: The blood of a guinea-pig which contains a rabbit serum component will elicit a contraction when added to the uterine preparation of an animal sensitized to rabbit serum; that is, a uterus the cells of which possess antibodies to rabbit serum.

The detailed description of one such experiment will demonstrate the mechanism of the method. A preliminary tracing is necessary to show that the uterus to be used in the experiment proper does actually respond actively to rabbit serum. Guinea-pig 1260 was injected 2½ months previously with 0.5 c.c. rabbit serum. The animal was killed and one horn of the uterus was placed in a Dale apparatus (Fig. 9). To this uterus 0.01 c.c. rabbit serum was added. The reaction indicates the presence of antibodies to rabbit serum in the uterus of Guinea-pig 1260. The other horn of this animal's uterus was used for the test.

The preparation of the animal which was to be tested for circulating antigen, was as follows: Guinea-pig 121 received 3 c.c. of rabbit serum vs. egg albumin, intraperitoneally. After an interval of 11 days the animal was exsanguinated and 1 c.c. reserved for this experiment. The second horn of the uterus of Guinea-pig 1260 was placed in a Dale apparatus. To this 0.5 c.c. of serum (Guinea-pig 121) was added (Fig. 10). The reaction indicates the presence of a rabbit serum component (antigen) in the serum of Guinea-pig 121.

COEXISTENCE OF FACTORS IN DESENSITIZATION

The second type of experiment which was mentioned as not being essential to the present study, but which is nevertheless of importance, involves the demonstration of the coexistence of cellular antigen and cellular antibody in partial desensitization.

The rationale of this procedure is as follows: A guinea-pig is sensitized to rabbit serum, and after an interval of several weeks, the desensitizing injection is given. However, the antigen used for this 2nd injection is itself an immune body. Depending on the conception of the interaction of antigen and antibody, two possibilities present themselves. The substance which is present in excess will completely neutralize the other, and either antigen alone or antibody alone will be demonstrated. Or, the two factors—antigen and antibody—will exist together. The latter has proved to be the correct view.

A detailed description of an experiment will illustrate the method.

Guinea-pig 363 was injected with 0.5 c.c. rabbit serum intraperitoneally. After an interval of 10 weeks the guinea-pig received the desensitizing injection; namely, 1 c.c. rabbit serum (Rabbit 291 highly immunized to egg albumin) intraperitoneally (Fig. 11). Two days later the guinea-pig was killed and the uteri were suspended in a Dale apparatus. The uterus contracted on the addition of egg albumin and of rabbit serum, thus indicating the presence in the cells both of rabbit serum (the antigen) and of antibodies to rabbit serum (the antibody) simultaneously. This result agrees with Weil's findings.

THEORETICAL CONSIDERATIONS

The entire period of reaction covered by these studies is 4 weeks. At the end of that time demonstrable antigen has disappeared from the cells and the blood. Antibody at the end of this period is present in large amount, both in cells and blood. But the complete evolution of the phenomenon is even now not perfected. During the succeeding periods there would be demonstrable a gradual fall in the antibody content in the blood, until a level is reached at which available methods would fail to reveal it. Coincidently, there is a drop in the cellular antibody, which, however, persists, according to Rosenau and Anderson, in sufficient amount to mediate anaphylactic shock for a period of 3 years—practically during the rest of the animal's life.

Hence, the mechanism of reaction to the injection of a foreign protein is a very complex phenomenon. In the present study the primary injection was rather large, namely, 3 c.c. There is every reason to believe that with smaller doses an essentially similar mechanism comes into play, altho it is readily conceivable that the time relationship of the various factors would present material differences.

The application of these data to infectious disease is necessarily imperfect because of the inadequacy of our knowledge with respect to the 4 factors concerned. There are certain facts, however, which indicate a similar mechanism. For instance, in typhoid fever, it is well

known that in a very high percentage of the cases blood cultures are positive early in the disease, while they tend to become negative in increasing degree as the disease progresses. The agglutination test for typhoid bacilli, however, pursues an inverse course; it is negative, as a rule, in the early stages, and becomes positive in a large proportion of cases as the disease progresses. The external reaction, as shown by Gay[8] and Austrian,[9] takes a course similar to that of the agglutination test in the blood. If, now, we consider the bacillus as the antigen, the agglutination test as evidence of circulating antibody, and the skin reaction as evidence of cellular antibody, it is evident that in general the mechanism presents striking analogies with those experimentally demonstrated. As an actual fact, the same patient may simultaneously present a positive blood culture and a positive agglutination test. It is freely admitted that this comparison is to some degree hypothetic as regards the identification of the several factors, but it presents suggestive features which may be of value in the understanding of infectious diseases. No less interesting is the observation recently made in a study of typhus fever, that during the early period of convalescence the blood may contain the typhus organism at the same time that it contains agglutinins thereto (Plotz, Olitzky, and Baehr[10]). In the same way Weil has shown that the injection of a large amount of therapeutic horse serum in a case of meningitis was followed by a period during which both the horse serum and antibodies thereto were demonstrable in the blood. At the present time the data bearing on this subject are extremely meager, but it seems likely that they will eventually accumulate in sufficient amount to throw new light on the reaction to infections.

CONCLUSIONS

Antigen both in the cells and in the blood, and antibody, likewise, in the cells and in the blood, may be demonstrated during a period of 3 weeks succeeding the injection of a foreign serum into a guinea-pig.

Antigen in the cells has been demonstrated for a period of 17 days after injection; antigen in the blood also for 17 days. After this time these factors apparently disappear.

Antibody is demonstrable in the cells from the 9th day onward. Antibody is demonstrable in the blood after the 14th day.

[8] Publ. in Path. Univ. of California, 1913, 2, p. 127.
[9] Bull. Johns Hopkins Hosp., 1912, 23, p. 1.
[10] Jour. Infect. Dis., 1915, 17, p. 52.

The interrelations of these 4 factors are probably very complicated. For a period of several days all may coexist in the body.

The observation is confirmed that after partial desensitization both antigen and antibody are demonstrable in the cell.

EXPLANATION OF PLATES 24 AND 25

PLATE 24

FIG. 1. A control experiment showing that 3 c.c. of egg albumin and 0.5 c.c. of rabbit serum produce no contraction when added to Locke's fluid surrounding a normal uterus.

FIG. 2. A control experiment showing that 5 c.c. of egg albumin do not produce a contraction in a normal uterus. The reaction to ergamine indicates the excellent contractility of this uterine muscle.

FIG. 3. The coexistence of antigen and antibody in the cell 9 days after injection. Guinea-pig 104 received, intraperitoneally, 2 c.c. of rabbit serum (Rabbit 291 highly immunized against egg albumin). After 9 days the guinea-pig was killed. Uterus responded to the addition of 0.1 c.c. egg albumin (thus indicating the presence of antigen) and to 0.1 c.c. rabbit serum (thus indicating the presence of antibody).

FIG. 4. The presence of antigen and antibody in the cell 11 days after injection. On October 17 Guinea-pig 121 received, intraperitoneally, 3 c.c. rabbit serum (Rabbit 291 highly immunized against egg albumin). After 11 days the animal was exsanguinated. The uteri were removed and tracings made. The response to 0.4 c.c. egg albumin indicates the presence of rabbit serum in the uterine cells (antigen) and the response to 0.3 c.c. rabbit serum, of antibodies to rabbit serum (antibody). The minimal contractions after the subsequent addition of 0.7 c.c. egg albumin and 0.6 c.c. rabbit serum indicate complete desensitization resulting from the first doses.

FIG. 5. The coexistence of antigen and antibody in the cell, 17 days after injection. On January 27, Guinea-pig 525 received, intraperitoneally, 3 c.c. of rabbit serum (Rabbit 564 highly immunized against egg albumin) ; 17 days later the animal was exsanguinated. The uteri were placed in a Dale apparatus and tested for antigen and antibody. The reaction to 1.8 c.c. egg albumin indicates the persistence of rabbit serum (antigen), and the response to 0.4 c.c. rabbit serum, demonstrates antibodies to rabbit serum (antibody). The latter feature being of the routine type in actively sensitized guinea-pigs is not reproduced.

FIG. 6. The presence of antigen in the circulation, 9 days after injection. Guinea-pig 65 received 2 c.c. of serum recovered by exsanguination of Guinea-pig 104. (Nine days previously Guinea-pig 104 had received 2 c.c. of rabbit serum versus egg albumin.) After an interval of 3 days Guinea-pig 65 was killed and the uteri suspended in a Dale apparatus. The reaction to 0.8 c.c. egg albumin indicates the presence of a rabbit serum component (antigen), which represents the passive absorption of the injected serum of Guinea-pig 104.

FIG. 7. The persistence of antigen in the blood 11 days after injection. Guinea-pig 137 received 2.25 c.c. of serum (Guinea-pig 121) intraperitoneally. (Eleven days previously Guinea-pig 121 had received 3 c.c. of rabbit serum versus egg albumin.) After 2 days Guinea-pig 137 was killed, and tracings made. The response to 2 c.c. egg albumin indicates the presence of a rabbit

serum component, which represents the passive absorption of the injected serum (Guinea-pig 121). Also, the absence of a response on the addition of 0.5 c.c. rabbit serum (shown in the tracing) indicates that circulating antibody had not appeared on the 11th day.

PLATE 25

FIG. 8. The presence of antigen and antibody in the blood 17 days after injection. Guinea-pig 590 received 2.25 c.c. of serum of Guinea-pig 525. (Seventeen days previously Guinea-pig 525 had received 3 c.c. rabbit serum versus egg albumin, Fig. 5.) Four days later Guinea-pig 590 was killed and tracings made. The reaction to 1.8 c.c. egg albumin indicates the presence of rabbit serum (antigen) and the response to 0.5 c.c. rabbit serum demonstrates antibodies to rabbit serum (antibody). Thus Figs. 5 and 8 show the coexistence of all 4 factors in one animal. Fig. 5 demonstrates the simultaneous presence of cellular antigen and cellular antibody and Fig. 8 of circulating antigen and circulating antibody.

FIG. 9. Preliminary experiment showing the presence of antibodies against rabbit serum in the uterus of Guinea-pig 1260. Guinea-pig 1260 received 0.5 c.c. rabbit serum, intraperitoneally, on August 13. Two and one-half months later the animal was killed and the horns of the uterus were suspended in the Dale apparatus. The reaction to 0.01 c.c. of rabbit serum indicates the presence of rabbit antibodies in high concentration.

FIG. 10. The presence of antigen in the blood. The addition of 0.5 c.c. serum of Guinea-pig 121 to a uterine preparation (Guinea-pig 1260) causes a contraction. This indicates a rabbit serum component (antigen) in the serum of Guinea-pig 121, as rabbit antibodies had been demonstrated in the opposite horn of Guinea-pig 1260 (Fig. 9).

FIG. 11. The demonstration of the coexistence of antigen and antibody in the cell by the method of desensitization. Guinea-pig 363 received 0.5 c.c. rabbit serum, intraperitoneally, on August 13. Ten weeks later 1 c.c. of rabbit serum (Rabbit 291 highly immunized against egg albumin) was injected intraperitoneally. After 2 days Guinea-pig 363 was killed and tracings made. Response to egg albumin indicates the presence of rabbit serum (antigen) and the response to rabbit serum indicates the persistence of antibodies to rabbit serum (antibody).

PLATE 24

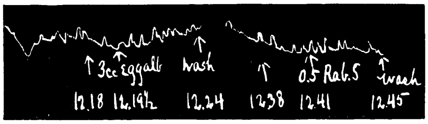

Figure 1

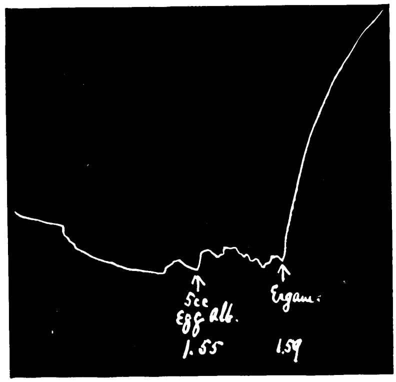

Figure 2

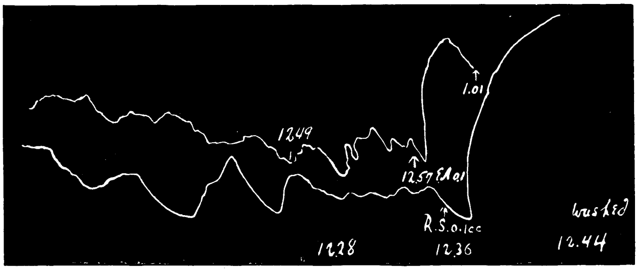

Figure 3

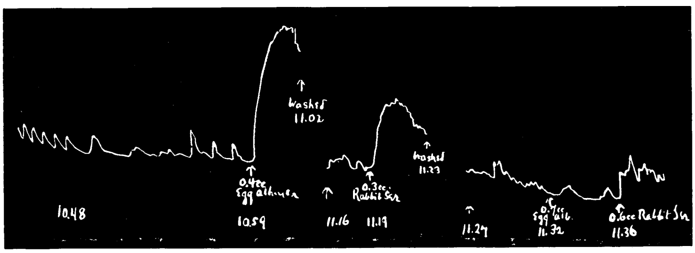

Figure 4

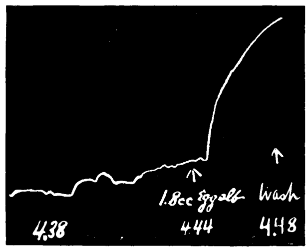

Figure 5

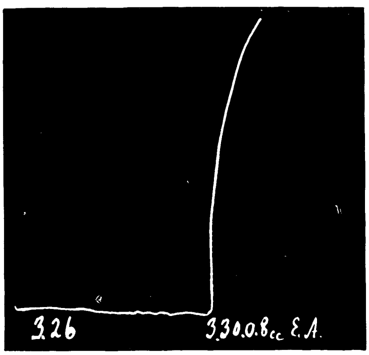

Figure 6

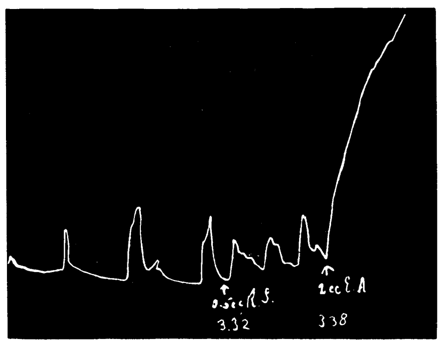

Figure 7

Plate 25

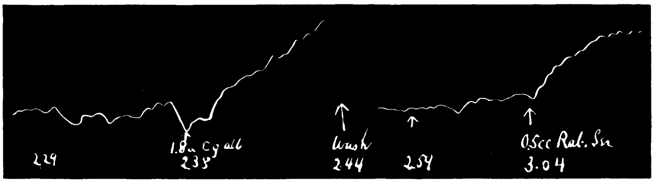

Figure 8

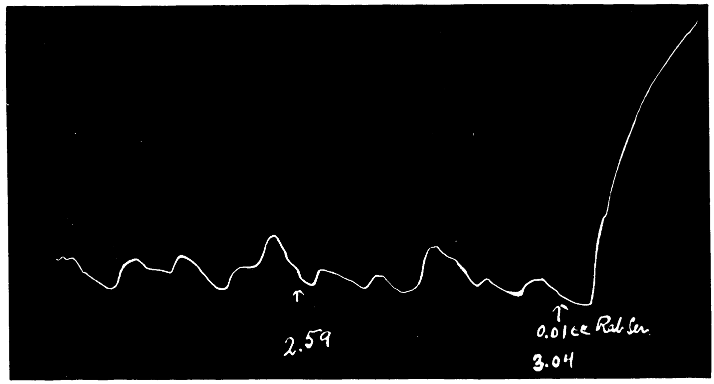

Figure 9

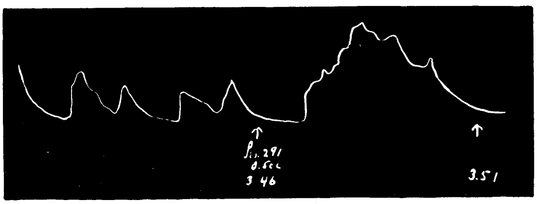

Figure 10

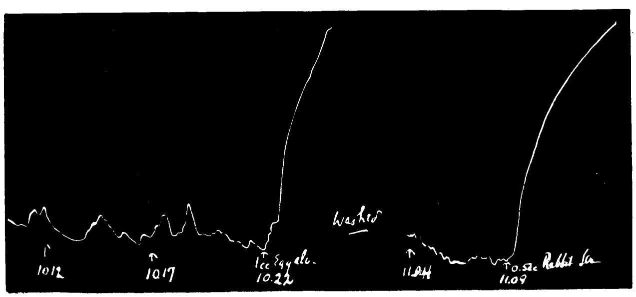

Figure 11